

Patent claims

1. An isolated polynucleotide from coryneform bacteria,
comprising a polynucleotide sequence which codes for
the menE gene, chosen from the group consisting of
- a) polynucleotide which is identical to the extent of
at least 70% to a polynucleotide which codes for a
polypeptide which comprises the amino acid sequence
of SEQ ID No. 2,
- b) polynucleotide which codes for a polypeptide which
comprises an amino acid sequence which is identical
to the extent of at least 70% to the amino acid
sequence of SEQ ID No. 2,
- c) polynucleotide which is complementary to the
polynucleotides of a) or b), and
- d) polynucleotide comprising at least 15 successive
nucleotides of the polynucleotide sequence of a),
b) or c),
- the polypeptide preferably having the activity of O-
succinylbenzoic acid CoA ligase.
2. A polynucleotide as claimed in claim 1, wherein the
polynucleotide is a preferably recombinant DNA which is
capable of replication in coryneform bacteria.
3. A polynucleotide as claimed in claim 1, wherein the
polynucleotide is an RNA.
4. A polynucleotide as claimed in claim 2, comprising the
nucleic acid sequence as shown in SEQ ID No. 1.
5. A DNA as claimed in claim 2 which is capable of
replication, comprising
- (i) the nucleotide sequence shown in SEQ ID No. 1, or

- (ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or
- (iii) at least one sequence which hybridizes with the sequence complementary to sequence (i) or (ii), and optionally
- (iv) sense mutations of neutral function in (i).
6. A DNA as claimed in claim 5 which is capable of replication,
wherein
the hybridization is carried out under a stringency corresponding to at most 2x SSC.
7. A polynucleotide sequence as claimed in claim 1, which codes for a polypeptide which comprises the amino acid sequences shown in SEQ ID No. 2.
8. A coryneform bacterium in which the menE gene is attenuated, in particular eliminated.
9. The integration vector pCR2.1menEint, which
- 9.1. carries an internal fragment of the menE gene 520 bp in size,
- 9.2. the restriction map of which is reproduced in figure 1, and
- 9.3. which is deposited in the E. coli strain Top10/pCR2.1menEint under no. DSM 14080 at the Deutsche Sammlung für Mikroorganismen und Zellenkulturen].
10. A process for the fermentative preparation of L-amino acids, in particular L-lysine,
which comprises
carrying out the following steps:

- a) fermentation of the coryneform bacteria which produce the desired L-amino acid and in which at least the menE gene or nucleotide sequences which code for it are attenuated, in particular eliminated;
- 5 b) concentration of the L-amino acid in the medium or in the cells of the bacteria, and
- c) isolation of the L-amino acid.
- 10 11. A process as claimed in claim 10, wherein bacteria in which further genes of the biosynthesis pathway of the desired L-amino acid are additionally enhanced are employed.
- 15 12. A process as claimed in claim 10, wherein bacteria in which the metabolic pathways which reduce the formation of the desired L-amino acid are at least partly eliminated are employed.
- 20 13. A process as claimed in claim 10, wherein the expression of the polynucleotide(s) which code(s) for the menE gene is attenuated, in particular eliminated.
- 25 14. A process as claimed in claim 10, wherein the catalytic properties of the polypeptide (enzyme protein) for which the polynucleotide menE codes are reduced.
15. A process as claimed in claim 10, wherein for the preparation of L-amino acids, coryneform microorganisms in which at the same time one or more of the genes chosen from the group consisting of
- 15.1 the dapA gene which codes for dihydrodipicolinate synthase,
- 15.2 the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase,

- 15.3 the tpi gene which codes for triose phosphate isomerase,
- 15.4 the pgk gene which codes for 3-phosphoglycerate kinase,
- 5 15.5 the zwf gene which codes for glucose 6-phosphate dehydrogenase,
- 15.6 the pyc gene which codes for pyruvate carboxylase,
- 10 15.7 the mqo gene which codes for malate-quinone oxidoreductase,
- 15.8 the lysC gene which codes for a feed-back resistant aspartate kinase,
- 15.9 the lysE gene which codes for lysine export,
- 15 15.10 the hom gene which codes for homoserine dehydrogenase
- 15.11 the ilvA gene which codes for threonine dehydratase or the ilvA(Fbr) allele which codes for a feed back resistant threonine dehydratase,
- 20 15.12 the ilvBN gene which codes for acetohydroxy-acid synthase,
- 15.13 the ilvD gene which codes for dihydroxy-acid dehydratase, and
- 15.14 the zwal gene which codes for the Zwal protein
- 25 is or are enhanced or over-expressed are fermented.
16. A process as claimed in claim 10, wherein for the preparation of L-amino acids, coryneform microorganisms

in which at the same time one or more of the genes
chosen from the group consisting of

- 16.1 the pck gene which codes for phosphoenol
pyruvate carboxykinase,
- 5 16.2 the pgi gene which codes for glucose 6-
phosphate isomerase,
- 16.3 the poxB gene which codes for pyruvate oxidase,
and
- 16.4 the zwa2 gene which codes for the Zwa2 protein
- 10 is or are attenuated are fermented.
- 17. A coryneform bacterium which contains a vector which
carries parts of the polynucleotide as claimed in
claim 1, but at least 15 successive nucleotides of the
sequence claimed.
- 15 18. A process as claimed in one or more of the preceding
claims, wherein microorganisms of the species
Corynebacterium glutamicum are employed.
- 19. A process for discovering RNA, cDNA and DNA in order to
isolate nucleic acids, or polynucleotides or genes which
20 code for O-succinylbenzoic acid CoA ligase or have a high
similarity with the sequence of the menE gene, which
comprises employing the polynucleotide comprising the
polynucleotide sequences as claimed in claims 1, 2, 3 or 4
as hybridization probes.